Counterstrategies by female frogs to sexual coercion by heterospecifics

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In mating systems with intense male–male competition for mates, males may coerce females into matings that impose fitness costs. Females able to alleviate negative fitness effects of sexual coercion by enforcing their mating preferences should have a selective advantage. What behaviours females of externally fertilizing species use to reduce costs of coercion, and how effective these behaviours are, is largely unknown. We experimentally evaluated mechanisms of mate choice in a system where indiscriminate and genetically nonmatching heterospecific males coerce females into matings and females are apparently passive participants of the mating game. We performed experiments using two frog species (Rana dalmatina and R. temporaria) which are sympatric and are often observed in heterospecific matings but do not produce viable offspring. We paired R. dalmatina females with a conspecific or a heterospecific male, placed pairs together with unmated R. dalmatina males and monitored female behaviour. Females paired with heterospecific males did not try to attract the attention of conspecific males, but they delayed egg laying. Females exerted cryptic female choice by laying fewer eggs when paired with heterospecific males. Finally, some females laid a small clutch of eggs, apparently to increase the likelihood of being released by their heterospecific mate and subsequently mate with a conspecific male. Female R. dalmatina thus have subtle but effective means to avoid the complete loss of a year's reproductive effort. In a broader context, females may be able to enforce their mating preferences even in externally fertilizing species where direct female choice is overrun by male–male competition.
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Sexual coercion by males is widespread in the animal kingdom (Clutton-Brock & Parker 1995), because in the majority of species males gain more by mating repeatedly (Bateman 1948), invest less in offspring (Trivers 1972), are more limited in the number of available mates (Emlen & Oring 1977) and have higher intrinsic maximum reproductive rates (Clutton-Brock & Parker 1992). Females often suffer high costs from sexual coercion in the form of increased energy expenditure and exposure to predation, injury or even death (Clutton-Brock & Parker 1995), but coercion may also reduce fitness of females by limiting their ability to choose between potential mates (Qvarnström & Forsgren 1998). Selection arising from this type of sexual conflict may favour females that can circumvent male control and are able to manipulate the outcome of a mating event to their own benefit.

Selection favouring females that succeed in enforcing their interests when facing sexual coercion may be especially strong when coercion occurs between heterospecifics. In this case, coercing males are generally incompatible with females and this may result in low fertilization success, low offspring quality or offspring nonviability (e.g. Kruuk et al. 1999; Pfennig & Simovich 2002; Valero et al. 2008). We may thus expect to find counterstrategies to sexual coercion in species pairs or groups that interfere during reproduction (Gröning & Hochkirch 2008) and such systems should provide excellent opportunities for studying the behavioural responses to coercion. Also, theory predicts that reproductive interference can not only have severe consequences for individuals but also affect the persistence of whole populations or even species (Kuno 1992; Rhymer & Simberloff 1996; Hochkirch et al. 2007). Despite their potential significance for behavioural ecology, evolutionary biology and conservation, behavioural counterstrategies to coercive reproductive interactions between heterospecifics have remained largely unexplored (Gröning & Hochkirch 2008).

Studies on within-species sexual coercion have proposed a number of behavioural mechanisms that females may use to reduce its costs. To decrease levels of sexual harassment, females may avoid areas with high male abundance (Parker 1970), form shoals (Pilastro et al. 2003; Dadda et al. 2005), forge coalitions (Smuts & Smuts 1993), or associate with territorial (Clutton-Brock

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et al. 1992) or dominant males (Clutton-Brock et al. 1988). Once paired, females may delay egg deposition, sperm transfer or actual fertilization (Clutton-Brock & Parker 1995), incite male–male competition (Cox & Le Boeuf 1977; Wiley & Poston 1996; Hoi 1997; Hoi & Hoi-Leitner 1997) or induce sperm competition (Smith & Reichard 2005) if this enhances the chances of their ova being sired by a superior male. Females may exercise cryptic female choice by selecting sperm from specific males (Pizzari & Birkhead 2000), by adjusting their investment in the production of a clutch and in the amount of parental care they provide according to the quality of their mates (Eberhard 1996). Females may also deceive males to make them invest more in reproduction (sensu Smith et al. 2007) and parental care (sensu Valera et al. 1997) or to prevent infanticide (Zinner & Deschner 2000).

We only know of one study explicitly testing for behavioural counterstrategies to sexual coercion by heterospecifics in externally fertilizing species, where several mechanisms (e.g. postcopulatory mate choice) are not feasible and, consequently, different behaviours from those in internal fertilizers may be important (Clutton-Brock & Parker 1995; Eberhard 1996; Birkhead 1998; Gil et al. 1999). Reyer et al. (1999) found that female waterfrogs (Rana lessonae and Rana erculenta) laid a smaller clutch when amplexed by undesired, sexually parasitic males, while the reduction in the number of spawned eggs increased the female's residual reproductive value. Further studies on anuran amphibians have proposed that females may defer pair formation with coercive, nonmatching and generally indiscriminate males (Emlen 1976; Robertson 1986; Bourne 1992). Females may approach conspecific males or, when a heterospecific male comes close to them, they may flee (Abt & Reyer 1993) or assume a vertical body position in an attempt to hinder amplexus formation (Emlen 1976). Once amplexed, females may delay egg deposition to increase the likelihood of the amplexed male losing interest in them or another male attempting to displace the already amplexed one (Hettyey & Pearman 2003) or assisting in the displacement of undesired males. This can be achieved by producing release calls or behaving conspicuously in front of other, more suitable males to incite male–male competition (Emlen 1976; Davies & Halliday 1977; Abt & Reyer 1993). However, there is little information on what behaviours females indeed use and whether these behaviours raise the chances of mating with preferred mates.

Using externally fertilizing anurans, we aimed to determine whether females are able to discriminate between coercive conspecific and heterospecific males, look for behavioural mechanisms that females already in amplexus may use to lessen negative fitness effects of sexual coercion and investigate the effectiveness of these counterstrategies to coercion. We used the Rana dalmatina – R. temporaria species pair because heterospecific matings occur among members of these species (personal observation) as a result of overlapping breeding seasons and the indiscriminate coercive mating behaviour of males (Reading 1984; Hettyey & Pearman 2003; Hettyey et al. 2005). Eggs laid in heterospecific matings do not enter embryonic development (personal observation); this allowed us to quantify costs of coercion and any fitness benefits arising from behavioural responses of females to coercion attempts.

METHODS

The Study Species

Rana dalmatina (RD) and R. temporaria (RT) are closely related European brown frogs (Green & Borkin 1993). They have a largely overlapping European distribution with RD having a more southern distribution ranging from northern France to the southern Balkans and RT also being present in northern Fennoscandia (Nöllert & Nöllert 1992). Both frogs are medium sized with RD being somewhat smaller than RT (Nöllert & Nöllert 1992). They are explosive breeders (Wells 1977) and their reproductive periods often coincide both spatially and temporally. The operational sex ratio is, in both species, strongly male biased throughout the breeding season. At low densities, males are stationary and call from territories (RD) or a chorus (RT), whereas at high densities intense scramble competition for mating opportunities arises in the form of prolonged wrestling and displacement attempts (Elmberg 1986; Ryser 1989; Lesbarrères & Lodé 2002; Lodé et al. 2005). As reported for other anuran species, intensive intrasexual competition may negatively affect fertilization success (Byrne & Roberts 1999) and lead to severe injuries or to the drowning of the female (Davies & Halliday 1979; Howard 1980; Hedengren 1987). Females are unable to repel mating attempts or terminate amplexus themselves. Males mate indiscriminately, which often leads to heterospecific matings in both directions (RD male with RT female and RT male with RD female, personal observation).

Experimental Procedures

We collected animals from two populations in the Pilis Mountains, Hungary (47°42′N, 19°02′E and 47°44′N, 19°01′E) at the beginning of the breeding season in March 2008. We captured males and females by hand while randomly searching the breeding ponds after dawn. We transported frogs to the site of the experiments located next to a third pond (47°43′N, 19°02′E), frequented by small populations of RD and RT. We kept individuals separated by sex in 35-litre plastic boxes filled with fresh pond water until the start of the experiment. We captured a total of 40 RD females, 140 RD males and 20 RT males. For each individual, we measured snout to vent length with a plastic ruler (± 1 mm) and body mass with a digital scale (± 0.1 g) and marked males for individual recognition with numbered waistbands. These consisted of a thick yarn bound around the waist of males and a 1×1 cm piece of self-adhesive tape stuck onto it. Waistbands did not seem to affect the behaviour of males. We removed waistbands before releasing males at the end of experiments.

We ran experimental trials in 20 plastic wading pools (80 cm diameter, 40 cm deep) containing ca. 15 cm of pond water, and a handful of sedge leaves providing cover and substrate for egg deposition. We assigned three RD males, one RD female and either a fourth RD male or an RT male to each container. The size of anuran breeding populations is highly variable over time, resulting in varying ratios of RT and RD at breeding sites. In the breeding ponds from which we collected animals, we have observed species ratios fluctuating between 1:2 and 1:20 (RT:RD) over the last decade (personal observation). Consequently, the ratio of male types in our experiment simulated conditions at the breeding site, where RD males usually largely outnumber RT males (Hettyey et al. 2003; personal observation). Density was also within the range that can be found in nature. We ran two consecutive rounds of trials, both started a few hours after dusk by placing three RD males into each wading pool and putting one RD female together with either a fourth RD male or an RT male into a covered plastic box (48×35 cm and 25 cm high, containing ca. 15 litres of pond water) placed next to each wading pool. Once amplexus had occurred, we moved the pair from the box to the wading pool containing three RD males. This allowed us to control species composition in initial pairs. Egg deposition never occurred in the plastic boxes or sooner than 8 h after we moved the pairs to the wading pools. We monitored each experimental unit every 20 min during the first hour and then every hour for the rest of the experiment. To collect information on whether a female was inactively hiding on the bottom or actively swimming around on the water surface, we noted vertical and horizontal positions of the female. We also

determined whether amplexus had occurred, which male was in amplexus, and whether egg deposition had occurred. We approached experimental units quietly and used dim red head-lights at night to minimize disturbance to experimental animals.

When a female laid eggs, we removed the clutch and if the female was released by the amplexing male and then not amplexed again within 2 h, we terminated the trial, removed the frogs and released them back into the pond where they were captured. To increase sample size and be able to perform a second round of experimental trials, we terminated the first round after 115 h, 11 h after the last observed egg deposition event and 55 h after the last take-over. We ran the second round for 69.5 h; termination was 7 h after the last observed egg deposition event and 8 h after the last take-over.

When terminating a trial, we first weighed the whole clutch and then separated a few portions totalling about 100 eggs. We weighed and counted eggs in these portions, calculated an average egg mass and divided total clutch mass by average egg mass to obtain an estimate of total number of eggs laid. This method is readily used in studies of female fecundity in anurans and was applied to prevent developing eggs being injured by splitting up the whole clutch. To determine fertilization success, we transported the 100 weighed eggs to the laboratory, where we kept eggs in groups of 20 in 12×15 cm plastic containers with aerated recon-stituted soft water (American Public Health Association 1985) barely covering the eggs. When developing eggs were easily discernible from dead ones (Gosner stage 19–20; Gosner 1960), we counted the live embryos and used their proportion as an estimate of fertilization success. Embryos were released back into the pond where parents were captured. The Közép-Duna-Völgyi Környe-zetvédelmi, Természetvédelmi és Vízügyi Felügyelőség issued a permit to conduct experiments.

Statistical Analyses

From a total of 40 trials, three females were never amplexed in the covered plastic boxes, in two cases by an RD male and in one case by an RT male. These trials were terminated after 3 days and could not be used in the analyses. In two further trials amplexus with conspecific males yielded close to 0% fertilization and in another trial an apparent mating with a heterospecific male led to 74% fertilization. We did not observe egg-laying events directly in these trials and can only speculate that males were infertile or abandoned the female shortly before egg deposition in the first two cases and that the take-over we registered when we found the eggs actually happened before egg laying in the third case. As we cannot be sure what happened in these cases, we excluded these trials from analyses where male type involved in matings had to be known.

Our response variables were the proportion of monitoring events when the pair was hiding under water, the proportion of monitoring events when the pair had moved since the last moni-toring event, the time until egg deposition from formation of the amplexus leading to egg deposition, the occurrence of take-overs before egg deposition, the number of eggs laid and the occurrence of egg deposition.

When analysing the time spent hiding on the bottom and swimming activity of amplexed females, we used data collected at night, as during the day animals often responded to our approach by attempting to escape, and as breeding activity is highest at night. We further narrowed down the analysis to data collected on the second night, when animals have presumably become accustomed to the experimental conditions but most females have not yet laid their eggs. We excluded from this analysis six trials where take-overs happened during the designated time period to avoid

pseudoreplication arising from the nonindependent nature of data on the same females before and after take-overs. This resulted in 17 replicates for the first round of trials and 14 replicates for the second round of trials. As (1) the time spent hiding on the bottom was negatively correlated with swimming activity (Spearman correlation: $r_S = -0.48$, $N = 31$, $P = 0.006$), (2) time spent hiding on the bottom and swimming activity of the pair are both thought to be controlled by the female and (3) these behavioural traits together determine conspicuousness of the pair, we combined these variables via principal components analysis (PCA). The first component explained 81.1% of the total variance in the time females spent hiding on the bottom and in swimming activity and these variables loaded strongly onto the first component. The time spent hiding on the bottom was negatively correlated and swim-ming activity was positively correlated with PC1 scores ($r = -0.9$ and $r = 0.9$, respectively). We used the factor scores on PC1 as a combined measure of 'conspicuous behaviour'. To determine whether females behaved more conspicuously when amplexed by a heterospecific male, we performed a general linear model (GLM) ANCOVA with conspicuousness as the dependent variable, male type and group of trials entered as fixed factors and female body mass as a covariate. To test whether females behaving more conspicuously are more likely to have their mate replaced, we applied a logistic regression. We entered replacement of the male in amplexus as the binary response variable, male type and group of trials as categorical covariates and conspicuousness and female body mass as continuous covariates. We excluded trials where two clutches were laid as it is unclear whether take-overs following egg laying are induced by the conspicuousness of the females' behav-iour, by egg laying per se or by a decreased interest of the amplexed male.

We analysed time to egg laying with a GLM ANCOVA. We entered the duration of the amplexus leading to egg deposition as the dependent variable, male type and group of trials as fixed factors and female body mass as a covariate. To determine whether delaying egg deposition may serve to enhance the chances of successful take-overs, we performed a logistic regression with the occurrence of take-overs as the dependent variable, type of amplexing male as a categorical covariate and the time available for take-overs (the length of time the pair was together before egg deposition or termination of the trial) as a continuous covariate. We excluded cases of repeated egg laying from these two analyses as delaying egg deposition and laying eggs repeatedly may be different tactics used by females to cope with harassment by het-erospecific males.

We analysed the number of eggs laid by entering relative egg number (eggs/g female body weight) as the dependent variable, male type and round of trials as fixed factors and time until egg deposition as a covariate into a linear mixed model (LMM) ANCOVA. We also entered trial number nested within the round of trials as a random factor into the model to avoid pseudoreplication caused by repeated egg depositions within trials. Finally, we explored the pattern of repeated egg laying with a Fisher's exact test. We entered all two-way interactions into initial models and applied backward removal model selection. All tests were two tailed. Statistics were calculated using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.).

RESULTS

We did not observe females producing release calls in the experiment or in the natural population. When analysing how conspicuously the amplexed pair behaved (time spent hiding combined with swimming activity, see above), we did not detect an effect of either male type (GLM ANCOVA: $F_{1,29} = 0.63$, $P = 0.43$;

Fig. 1) or round of trials ($F_{1,29} = 2.52$, $P = 0.12$) or their interaction ($F_{1,27} = 0.39$, $P = 0.54$). Female body mass also had no effect ($F_{1,29} = 0.62$, $P = 0.44$). Conspicuous behaviour of the pair did not affect the likelihood of the amplexed male eventually being replaced by another male (logistic regression: Wald $\chi^2 = 1.63$, $N = 29$, $P = 0.2$). The likelihood of subsequent take-overs was also not affected by female body mass (Wald $\chi^2 = 0.51$, $N = 29$, $P = 0.47$), round of trials (Wald $\chi^2 = 0.29$, $N = 29$, $P = 0.59$), type of male in amplexus (Wald $\chi^2 = 0.29$, $N = 29$, $P = 0.59$) or any of the two-way interactions (all $P > 0.2$).

Time until egg deposition depended on male type with females laying eggs later when amplexed by heterospecific males than when amplexed by conspecific males (GLM ANCOVA: $F_{1,12} = 8.77$, $P = 0.012$; Fig. 2). Eggs were deposited in the second round of trials sooner than in the first round ($F_{1,12} = 26.88$, $P < 0.001$). The effect of female body mass was also significant ($F_{1,12} = 5.49$, $P = 0.037$). The interaction between male type and female body mass was marginally nonsignificant ($F_{1,11} = 3.51$, $P = 0.088$) whereas the other two-way interactions did not have an effect on the timing of egg laying (all $P > 0.2$). A logistic regression suggested that male type had an effect on the likelihood of take-overs (RD males were replaced in 13 of 17 cases, whereas heterospecific males were replaced in three of 14 cases; Wald $\chi^2 = 8.17$, $N = 31$, $P = 0.004$) and showed that delaying egg deposition did not have a significant effect overall (Wald $\chi^2 = 0.77$, $N = 31$, $P = 0.38$). The interaction between male type and timing of egg deposition, however, tended to influence the likelihood of take-overs (Wald $\chi^2 = 3.69$, $N = 31$, $P = 0.055$).

Females laid fewer eggs relative to their initial body mass when amplexed by heterospecific males than when mated with conspecific males (LMM ANCOVA: $F_{1,17} = 10.2$, $P = 0.005$; Fig. 3) and more eggs in the second round of trials ($F_{1,15} = 5.6$, $P = 0.032$). Females also deposited more eggs after being in amplexus with the male for a long time ($F_{1,15.9} = 7.64$, $P = 0.014$). The interaction between the time until egg deposition and male type was marginally nonsignificant ($F_{1,14.8} = 3.7$, $P = 0.075$). The other two-way interactions were nonsignificant ($P > 0.2$).
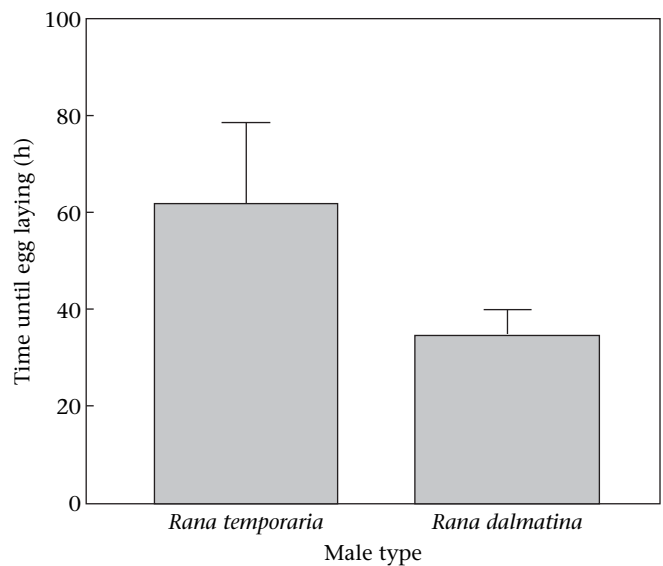

Figure 2. Duration of amplexi (mean ± 1 SE) leading to egg laying when *Rana dalmatina* females were amplexed by either a heterospecific or a conspecific male.

Finally, we observed repeated egg deposition in three of six cases where the female started egg deposition while amplexed by a heterospecific male, but we did not observe this behaviour in any of the 13 trials where females started to lay eggs while amplexed by a conspecific male. This pattern deviated significantly from a random distribution (Fisher's exact test: $\chi_1^2 = 6.5$, $P = 0.031$; Fig. 4). The pattern of repeated egg laying was as follows: after the female had laid 59, 296 and 785 eggs, the heterospecific male was replaced by a conspecific male within 2, 2 and 7 h and a second clutch of 880, 743 and 207 eggs was laid, respectively, within 1 h of take-over. In one further case, a heterospecific male remained in amplexus even after the female had laid 1089 eggs and 45 h later another 20 eggs. Owing to the very small size of the second clutch, we do not consider this as a case of repeated egg laying, but including it into the analysis would strengthen our results.
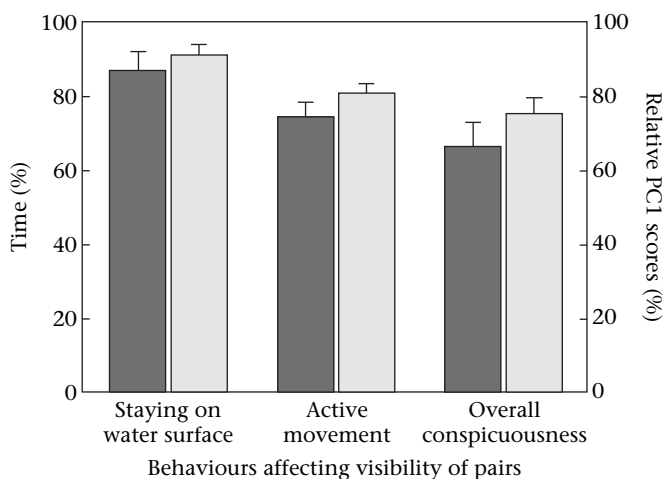

Figure 1. Time spent hiding on the bottom, swimming activity and a composite measure of conspicuousness of pairs (mean ± 1 SE). For easier visual understanding, hiding is presented as a percentage of time the pair spent on the water surface and swimming activity is presented as a percentage of time the pair had moved since the last observation. For graphical representation of overall conspicuousness we calculated percentage values from factor scores derived from a PCA on time spent hiding and swimming activity by adding the minimum value to each score and dividing it by the range. Black bars represent females amplexed by a heterospecific male; grey bars represent females amplexed by a conspecific male.
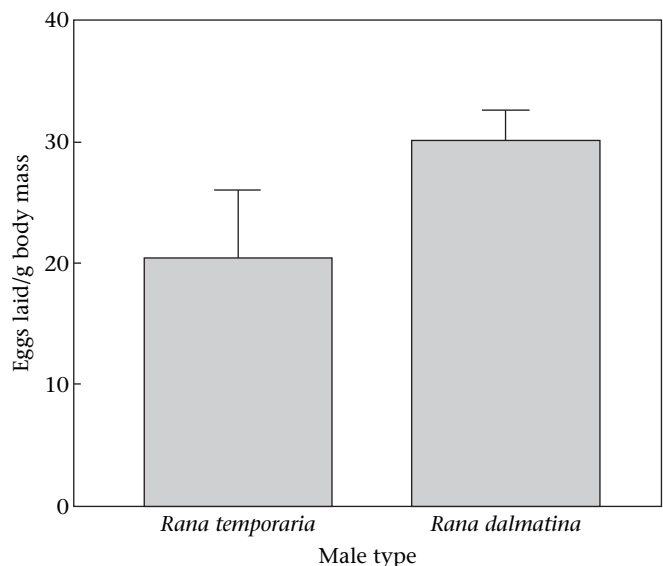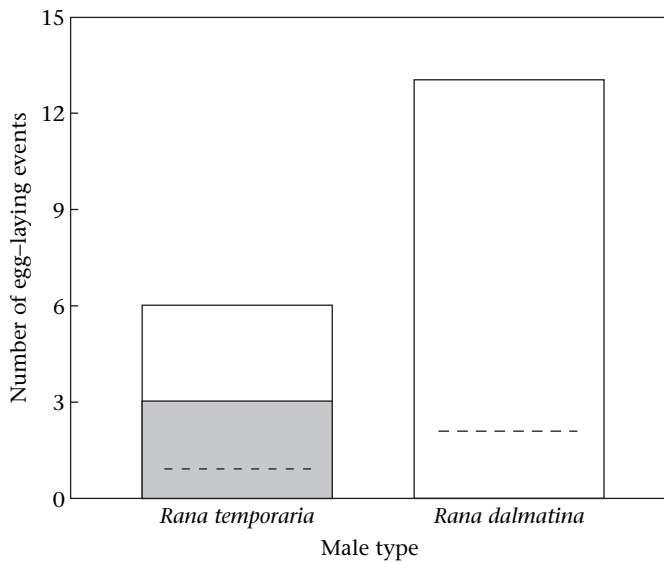

Figure 3. Relative egg numbers (mean ± 1 SE) laid by *Rana dalmatina* females mated with heterospecific or conspecific males. As egg number was strongly correlated with body size, we used the number of eggs per g body mass in the analyses.

Figure 4. Number of occasions when the *Rana dalmatina* female deposited eggs for the initially amplexing heterospecific or conspecific male. Bars represent the number of egg-laying events; grey indicates the events when repeated egg laying occurred. Dotted lines represent expected frequencies of repeated egg laying, assuming a random distribution.

DISCUSSION

Our results confirmed previous observations on the indiscriminate mating behaviour of RT males (Reading 1984), which is typical of many anuran species (e.g. *R. esculenta*: Engeler & Reyer 2001; *Bufo bufo*: Marco & Lizana 2002; *R. dalmatina*: Hettyey & Pearman 2003). *Rana temporaria* males readily amplexed RD females, remained in amplexus with them for several days if the female did not deposit eggs sooner and tenaciously defended their position against take-over attempts. We observed take-overs more often in cases where conspecific males were initially in amplexus than when heterospecific males were to be displaced. Consequently, as (1) females cannot remove the amplexing male from their back, (2) they cannot rely on the discriminative ability of heterospecific males, and (3) conspecific males are rarely able to dislodge heterospecific males from amplexus, the question is pertinent for the case of RD females: what behavioural mechanisms can RD females use to counter the threat of losing the reproductive output of an entire year when amplexed by a heterospecific male?

Females did not behave more conspicuously when amplexed by heterospecific males, that is, they did not spend more time on the water surface or show increased swimming activity to solicit male–male competition (sensu Cox & Le Boeuf 1977; Wiley & Poston 1996). Analyses performed on the two behavioural components separately yielded the same result. Also, females behaving more conspicuously did not seem to experience a higher chance of their mate being replaced. Even though conspicuous or cryptic behaviour may have more pronounced effects on visibility in a spatially more complex natural environment, our results suggest that female RD do not attempt to induce male–male competition when amplexed by nonmatching males as do females of some other taxa (Cox & Le Boeuf 1977; Hoi 1997; Hoi & Hoi-Leitner 1997; Pizzari 2001). Costs arising from increased predation or a higher likelihood of drowning by several simultaneously amplexing males (Davies & Halliday 1979) may not be offset by benefits arising from a slight increase in the probability of conspecific matings in RD females.

Females delayed egg deposition when amplexed by heterospecific males. A similar response to heterospecific amplexus in

another frog (Hettyey & Pearman 2003), and to harassment by undesired conspecific males in a fish (McGhee et al. 2007), suggests that delaying egg deposition may be a tactic widely used by females subject to sexual coercion in externally fertilizing species. When we tested whether delayed egg deposition may be considered an adaptive behavioural response, we observed different effects depending on male type. Delaying egg deposition raised the chances of take-overs when amplexed by conspecific males, but, probably because of the superior competitive ability of heterospecific (RT) males and a limited number of conspecific males present, this tactic did not help females amplexed by heterospecific males. The chances of delayed egg deposition resulting in take-overs may, however, be higher under natural conditions where amplexed pairs encounter a bigger population of males that is more likely to contain conspecific males with superior competitive abilities. Also, the male in amplexus may become exhausted by continuous attacks from other males or may switch to an alternative and more suitable female. Delaying egg deposition may thus indeed be an adaptive response to sexual coercion by undesired males.

Females laid approximately 30% fewer eggs for heterospecific males than for conspecific males. Clutch size adjustment by anuran females has previously been shown to occur as an adaptive response to multiple male amplexus lowering fertilization success (D'Orgeix 1996). Furthermore, Reyer et al. (1999) showed that female *R. lessonae* and *R. esculenta* responded to matings involving sexually parasitic hemiclonal males by laying fewer eggs. They also showed that this reduction in clutch size can lead to an increase in reproductive output through a second mating in the same year or through a larger clutch next year. The RD–RT system is similar to the *R. lessonae*–*R. esculenta* species pair, in that female choice is overrun by male–male competition and sexual coercion by indiscriminate males (Abt & Reyer 1993; Bergen et al. 1997; Engeler & Reyer 2001) and mating with the wrong male leads to no reproductive success at all (Uzzell et al. 1980). If we assume that lower egg numbers in one mating can be traded up for an increased clutch size in the next mating in RD as in the congeners *R. lessonae* and *R. esculenta*, our results also support the hypothesis that adaptive clutch size adjustment in response to sexual coercion may be widespread in anuran amphibians.

We observed three cases where females laid a clutch of considerable size soon after their mate was replaced. Repeated egg laying may be an extremely rare phenomenon in pure RD populations; Lodé et al. (2004) did not find any signs of it when they analysed 95 clutches in 11 ponds. Accordingly, we did not observe repeated egg laying in our experiment when the female was amplexed by a conspecific male. However, when the RD female laid eggs for a heterospecific male, repeated egg laying occurred in 50% of the cases. It thus appears that RD females may lay some of their eggs to manipulate the behaviour of heterospecific males and increase the chance of a successful take-over by a conspecific male. Reyer et al. (1999) also observed repeated egg laying, but it could not be interpreted as a behavioural response to coercion by nonmatching males as female *R. lessonae* and *R. esculenta* are known to lay their eggs in several clutches (Nöllert & Nöllert 1992) and repeated egg laying occurred in their experiment independent of male type in the first mating.

We may consider the observed egg-dumping behaviour of RD females as behavioural deception in the general sense, where senders behaviourally manipulate receivers to their own benefit: some females produced a small clutch apparently to imitate the completion of egg deposition and thus to increase the likelihood of the amplexing male being replaced and subsequently to be able to deposit withheld eggs when mated with a second male. Semple & McComb (1996), however, argued that we should only categorize

a certain behaviour as deceptive if it bears costs for the receiver. In the sense of this narrower definition, the observed egg-laying behaviour of RD females may not be termed behavioural deception as benefits to males most likely outweigh costs: RT males become released from a time-consuming and nonrewarding activity during an extremely short reproductive period and get the chance to find a conspecific female. None the less, it seems safe to suggest based on our results that females have manipulated male behaviour by laying some of their eggs.

The question arises, how important may the above female behaviours be under natural conditions? In the two populations where we collected animals, approximately 10% of observed amplexi were between heterospecifics. As RD greatly outnumbered RT in spring 2008 and observations during the past 5 years suggest
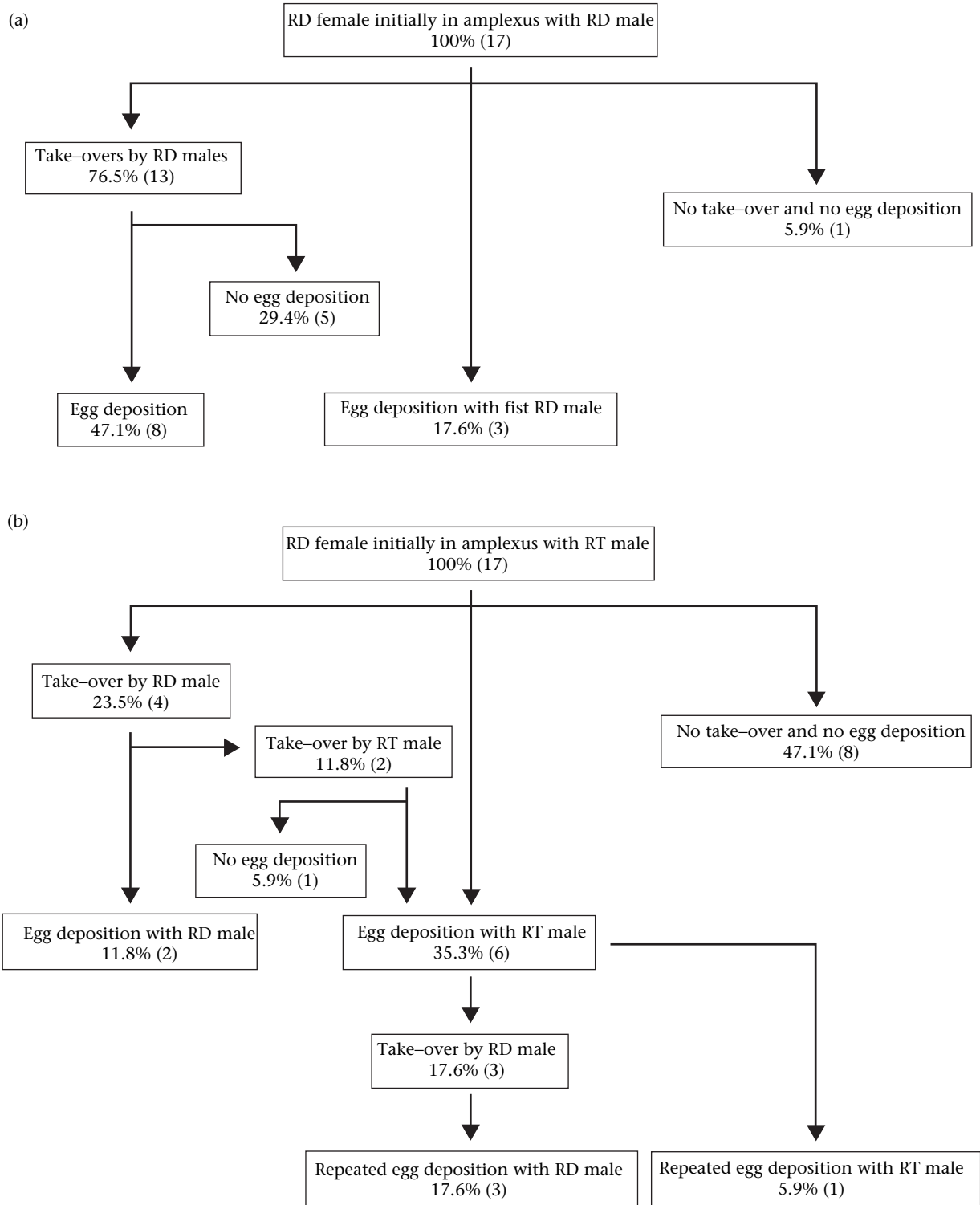
Figure 5. Summary of the experiments where (a) RD females were initially amplexed by a conspecific male and where (b) RD females were initially amplexed by a heterospecific male. We provide percentages calculated based on the number of trials where the female was amplexed and where we were sure which male was in amplexus at the time of egg laying (for details see text). Absolute numbers of trials are given in parentheses.

that relative numbers of the two species may be even greater in other years, amplexi between heterospecifics may usually be more frequent. Consequently, a considerable proportion of the female RD population may be exposed to the risk of mating with a heterospecific male and losing the reproductive investment of an entire year. Our results suggest that to avoid this, some females may delay egg deposition to increase the likelihood of their heterospecific mate becoming replaced by a conspecific male. For this there was a chance of 23.5% in our experimental set-up (see Fig. 5). Other females may actively manipulate the behaviour of their genetically nonmatching mate by laying a partial clutch. This was successful in 50% of the cases where the female laid a clutch together with a heterospecific male and in 17.6% of the cases overall (see Fig. 5). Excluding trials where no egg deposition occurred, and thus considering only trials where we know the definite and final outcome, the female initially amplexed by a heterospecific male laid all of its eggs with a heterospecific male in 37.5% of the cases ($N = 3$ cases), laid all of its eggs with a conspecific male in 25% of the cases ($N = 2$ cases) and laid approximately half of its eggs with a conspecific and the other half with a heterospecific male in 37.5% of the cases ($N = 3$ cases). It remains hard to extrapolate from the results of this experimental study to the effectiveness of female counterstrategies under natural conditions, but these results indicate that such counterstrategies do exist and may considerably reduce the chances of females losing a whole year's reproductive effort because of sexual coercion by heterospecific males.

Another intriguing question is why male frogs do not discriminate against heterospecific females. Males able to distinguish between conspecific and heterospecific females would clearly be at a selective advantage in mixed-species ponds since prolonged amplexus and mating may incur significant costs in terms of energy, sperm and missed mating opportunities (McLister 2003; Lengagne et al. 2007; Hettyey et al. 2009). We hypothesize that the ability to discriminate may not get past the early stages of preference evolution, when mistakes are frequently made, since costs of a mistaken refusal are likely to be higher than those of a mistaken acceptance in mating systems where the operational sex ratio is typically strongly male biased and mating opportunities are severely limited.

In summary, we investigated female behaviours that had been proposed to aid anuran females in countering the negative fitness effects of being amplexed by sexually coercive, heterospecific males. While experimental findings need careful validation under natural conditions (e.g. Hettyey & Pearman 2003, 2006; Ficetola & De Bernardi 2005), our results indicate that female RD may be able to distinguish between conspecific and heterospecific mates and may adaptively respond to them and avoid or minimize fitness loss caused by heterospecific matings. More generally, it seems that females may have subtle but effective means to express their mating preferences in species where male–male competition apparently overrules female mate choice. By increasing the fitness of individual females by alleviating the negative effects of sexual coercion by heterospecific males, the observed behavioural patterns may decrease the strength of reproductive interference and may ultimately contribute to the stable coexistence of syntopic species.

Acknowledgments

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